UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: February 6, 2014

SUBJECT: **FURFURAL and FURFURYL ALCHOHOL:** Report of the Cancer Assessment Review Committee

PC Code: 043301 and 643300
Decision No.: N/A
Petition No.: N/A
Risk Assessment Type: Cancer Assessment
TXR No.: 0056891
MRID No.: N/A

DP Barcode: N/A
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A

CAS No.: N/A
40 CFR: N/A

FROM: Jess Rowland, Co-Chair
Karlyn Middleton, Co-Chair
Cancer Assessment Review Committee
Health Effects Division (7509P)

TO: Elissa Reaves, Branch Chief (RABIV)
Health Effects Division (7509P)

The Cancer Assessment Review Committee (CARC) met on December 11, 2013 to evaluate the cancer classification of furfural and furfuryl alcohol in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

Furfural and Furfuryl Alcohol

PC CODES: 043301 and 643300

FINAL
FEBRUARY 6, 2014

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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EXECUTIVE SUMMARY

On December 11, 2013, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs evaluated the carcinogenic potential of furfural and furfuryl alcohol. Furfural is a pesticide active ingredient that is used to control root-infesting plant parasitic nematodes and fungal plant diseases and is currently registered for non-food use on athletic fields and outdoor ornamentals. In accordance with the 40 CFR Part 158 Toxicology Data requirements, long-term studies (i.e., chronic/carcinogenicity study in rats and the carcinogenicity study in mice) are not required for non-food use pesticides. The Agency has received a petition for a new use pattern (injection into soil) for pre-plant soil which may result in exposure via drinking water and thus there is a need for a dietary risk assessment. Consequently, toxicology and carcinogenicity studies conducted by the National Toxicology Program (NTP) were used to assess the carcinogenic potential of furfural/furfuryl alcohol. Additionally, all available data on toxicity, metabolism, mutagenicity, Structure Activity Relationships (SAR), and the report of the European Food Safety Authority (EFSA) on furfural derivatives were considered in this evaluation.

Male and female F344 N rats received oral (gavage) administration of furfural (99% pure) in corn oil at 0, 30 or 60 mg/kg/day for 104 weeks (MRID 46011016). Cholangiocarcinomas of the liver was seen in male rats at the high dose. The increase in the tumors incidences did not reach statistical significance when compared with the concurrent controls; however, historical control data indicate that this is a rare tumor and the incidences in this study (4%) were 10-fold higher than the historical control incidence (0.4%) for this tumor type. In addition, the presence of this tumor was corroborated by the occurrence of bile duct lesions which were histologically similar to the cholangiocarcinomas and were accompanied by fibrosis. These lesions were considered to be an early stage in the development of cholangiocarcinomas and thus could progress to this lesion. Also centrilobular necrosis of the liver occurred at increased incidences in the treated groups. The concern for this tumor was elevated since cholangiocarcinomas of the liver were also seen in male rats exposed to furan which is structurally related to furfural. Based on these considerations, the CARC concluded that the cholangiocarcinomas of the liver in male rats are treatment-related. The CARC considered the doses tested in both sexes to be adequate, but not excessive, to assess the carcinogenic potential of furfural. This determination was based on the results of the 16-day and 13-week studies which used for dose selection and the presence of non-neoplastic and neoplastic lesions in male rats in the main study.

Male and female B6C3F1 mice received oral (gavage) administration of furfural (99%) at 0, 50, 100 or 175 mg/kg/day in corn oil for 104 weeks (MRID 46011016). There were statistically significant ($p \leq 0.01$) increases in the incidences of hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas at the high dose in males. The increase in carcinomas was particularly note worthy, with the incidence in the high dose group being three-fold greater than the incidence in the vehicle control group. In female mice, incidences of hepatocellular adenomas increased with dose and were significantly ($p \leq 0.01$) greater in the high dose. The incidences of all of these tumors exceeded the reported historical control ranges. The tumors were also corroborated by the presence of non-neoplastic lesions characterized as multifocal pigmentation and chronic inflammation of the subserosa of the liver in both sexes of mice. The concern for this tumor type was elevated since liver tumors were also seen in male

rats exposed to 5-methylfurfural, which is structurally related to furfural. Therefore, the CARC considered the liver tumors in male and female mice to be treatment-related. The doses used in the mouse cancer study were considered to be adequate, but not excessive, in both sexes, to assess the carcinogenic potential of furfural. This was based on the results of the 16-day and 13-week studies used for dose selection, and the presence of non-neoplastic lesions in both sexes of mice in the main study.

Male and female F344/N rats were exposed to furfuryl alcohol (98% pure) by inhalation (vapor) at concentrations of 0, 2, 8 or 32 ppm for 104 weeks (MRID 49161601). Nasal cavity tumors were seen in both sexes of rats. Nasal epithelial squamous cell carcinomas were seen in 11% of male rats at the highest concentration compared to none in the chamber controls. There were also significant ($p \leq 0.01$) increases in combined nasal epithelial carcinomas and epithelial squamous cell carcinomas at the high dose (12%) compared to chamber controls (0%). Female rats only had a significant trend ($p < 0.05$) for nasal epithelial adenomas at the highest concentration. The occurrence of these tumors was corroborated by the presence of non-neoplastic nasal lesions observed in male and female rats. The incidences of hyperplasia of the lateral wall of the nasal cavity, atrophy and metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium were significantly elevated relative to controls at all three exposure concentrations in both sexes. The lesions increased in incidence and severity with increasing concentration. The CARC considered the nasal tumors in male rats to be treatment-related. The doses used in the rat study were considered to be adequate, but not excessive, in both sexes to assess the carcinogenic potential of furfuryl alcohol. This was based on the results of a subchronic inhalation study used for dose selection and the presence of non-neoplastic lesions in both sexes in the main study.

Male and female B6C3F1 mice were exposed to furfuryl alcohol (98% pure) by inhalation (vapor) at concentrations of 0, 2, 8 or 32 ppm for 104 weeks (MRID 49161601). Kidney tumors were seen in male mice. There were statistically significant increases for renal adenomas (6%; trend at $p \leq 0.01$), carcinomas (4%) and combined adenomas/carcinomas (10% at $p \leq 0.05$) at the high dose when compared to controls (0%). Kidney tumors are rare among the historical controls and the incidences of the combined tumors in this study (10%) were 25-fold higher than the historical control incidence (0.4%). The tumors were corroborated by the presence of non-neoplastic kidney lesions in both male and female mice. The severity of the lesions increased with increasing dose in male mice only. Therefore, the CARC considered the kidney tumors in male mice to be treatment-related. The doses used in the mouse cancer study with furfuryl alcohol were considered to be adequate, but not excessive, in both sexes to assess the carcinogenic potential of furfuryl alcohol. This was based on the results of a subchronic inhalation study used for dose selection, and the presence of non-neoplastic lesions in both sexes and neoplastic lesions in males in the main study.

Based on the available NTP genetic toxicology data, there is no mutagenic concern for furfural or furfuryl alcohol. Overall, the data for furfural suggest that it has intrinsic mutagenic potential in cultured mammalian cells. However, it is not expressed in whole animals since it is rapidly metabolized by the liver and rendered either non-mutagenic or markedly less mutagenic. Additionally, the negative data for the *in vivo* gene mutations assay, which examined the mouse liver as the target for furfural-induced tumorigenic activity, rule out mutagenicity as a possible

mode of action for the induction of liver tumors seen in the 2-year mouse bioassay. Furfuryl alcohol is not mutagenic in bacteria and does not cause chromosome aberrations or sister chromatid exchange induction in mammalian cells. The *in vitro* data are supported by the results of whole animals studies showing that furfuryl alcohol was not clastogenic, aneugenic or genotoxic in mouse bone marrow cytogenetic, micronucleus or SCE assays.

An analysis of structure activity relationships for furfural and furfuryl alcohol, provide additional support for the carcinogenic potential of furfural. A rare tumor type (cholangiocarcinomas of the liver) seen in rats exposed to furfural was also seen in rats exposed to furan. Similarly, liver tumors seen in male mice with furfural were also seen in mice exposed to 5-methylfurfural. Being a direct-acting reactive electrophilic chemical, furfural is expected to have a greater potential for inducing cancer by the inhalation route than the oral route because of the portal-of-entry effect. A structurally related chemical, formaldehyde, is a well-known carcinogen via the inhalation route but does not seem to be have a cancer concern by the oral route unless there is a massive exposure to overwhelm the detoxification capacity. There is some suggestive evidence that the nasal carcinogenic effect of furfuryl alcohol via the inhalation route may be related to its oxidation to furfural as the proximate or ultimate carcinogen. Because of these concerns, the CARC recommends a chronic toxicity/carcinogenicity study in rats by the inhalation route be conducted for furfural.

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005): **Furfural is classified as "Likely to Be Carcinogenic to Humans"** based on the following considerations:

(i) Treatment-related cholangiocarcinoma of the liver, a rare tumor type, observed in male rats;
(ii) Treatment-related liver tumors (adenomas, carcinomas and/or combined adenomas/carcinomas) observed in male and female mice; and
(iii) Occurrence of hepatocellular neoplasms in each sex of mice with compounds structurally very similar to furfural

Furfuryl alcohol as "Likely to Be Carcinogenic to Humans" based on the following consideration:

(i) Treatment-related nasal tumors (adenomas, carcinomas and/or squamous cell carcinomas observed in male rats): and
(ii) Treatment-related kidney tumors (adenomas, carcinomas and/or combined adenomas/carcinomas observed in male mice.

The CARC recommended the low dose extrapolation method (Q_1*) for quantification of human cancer risk since no mode of action studies are available for the tumor types seen in animals treated with furfural or furfuryl alcohol.

I. INTRODUCTION

On December 11, 2013, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of furfural and furfuryl alcohol.

II. BACKGROUND INFORMATION

Furfural is an aromatic aldehyde that is currently registered for non-food use on athletic fields and outdoor ornamentals. The current action is requesting a new use pattern (injection into soil) for pre-plant soil. Furfural (Figure 1) is a pesticide active ingredient that is used to control root-infesting plant parasitic nematodes and fungal plant diseases. The pesticidal mode of action is an interaction with the cuticle of the nematode, resulting in a stripping of the protective layers which results in the cuticle swelling and disintegration. This leads to decreased movement of the nematode and it subsequently dies through dehydration or attack by parasitic organisms. The mammalian mode of toxic action has not been identified. In its natural state, furfural is a liquid with high water solubility (s ≥ 74,100 ppm, FAO solubility classification) and is miscible with a variety of common organic solvents. Furfural has a relatively high vapor pressure and a moderately high Henry's Law constant (2.5 mm Hg and 4.4 x 10^{-6} atm-m^3/mol, respectively). Its C_{water}/C_{air} is equal to 5,812 (unitless), and its K_{AW} is 1.72x10^{-4}. These values indicate that furfural is slightly volatile from a water surface. The major furfural degradates, 2-furoic acid and furfuryl alcohol (Figure 2) may also be somewhat volatile (vapor pressures of 0.103 and 0.609 mmHg, respectively). Furfuryl alcohol is miscible with water. The octanol/water partition coefficients for furfural, 2-furoic acid and furfuryl alcohol 1 are very low (K_{OW} = 2.2, 4.4 and 1.9, respectively), indicating a very low tendency to bioaccumulate/bioconcentrate.

Figure 1. Structure of furfural.

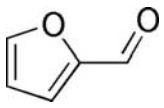
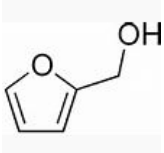


Figure 2. Structure of furfuryl alcohol.



III. EVALUATION OF CARCINOGENICITY STUDIES

At the present time, furfural is registered for non-food use on athletic fields and outdoor ornamentals. Since long-term toxicity and carcinogenicity studies are not required for non-food use chemicals, none were submitted to the Agency. However, the request for a new use may result in exposure via drinking water and thus a dietary assessment is required. Consequently, the toxicology and carcinogenicity studies with furfural conducted by the National Toxicology Program (NTP) will be used to evaluate the carcinogenic potential of furfural following oral (gavage) exposure (MRID 46011016) and furfuryl alcohol following inhalation exposures (MRID 49161601).

1. Carcinogenicity Study in Rats with Furfural

Reference: NTP (National Toxicology Program). 1990. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Furfural (CAS No. 98-01-1) in F344/N rats and B6C3F1 mice (Gavage Studies). NTP-TR-382, MRID 46011016.

A carcinogenicity oral gavage study in F344/N rats was conducted by the NTP. In this study, F344/N male and female rats (50/sex/dose) were treated via gavage with 0, 30, or 60 mg/kg bw/day, in corn oil, 5 days/wk for 2 years (104 weeks). Rats were 7-8 weeks of age at the start of the study.

Survival data and tumors were evaluated by the Office of Pesticide Programs Health Effects Division the results of which are presented below. For further details, see TXR 0056717, Memorandum from L. Brunsman to A. Howard (July 25, 2013).

A. Experimental Design- Rat Study

Furfural was administered in corn oil to F344/N rats (50/sex/dose) at dose levels of 0, 30, or 60 mg/kg bw/day, 5 days/wk via oral gavage for 2 years (104 weeks) in a combined chronic toxicity/carcinogenicity study.

B. Discussion of Survival Data- Rat Study

Although 21 male rats died of accidental death (6 in the control group, 7 at 30 mg/kg/day and 8 at 60 mg/kg/day), there were no statistically significant survival disparities among the dose groups for male rats (Table 1). No statistical evaluation of survival was performed on the female rats, but 38% (19/50) of the female rats died of accidental death in the 60 mg/kg/day dose group. In comparison, there were only 4 accidental deaths in the female rats in the control group, and only 2 accidental deaths at 30 mg/kg/day. However, the mortality observed at the high dose was not considered excessive and the doses used in female animals were adequate for assessing carcinogenicity.

Table 1. Furfural – F344/N Rat Study Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	Weeks				
	1-26	27-52	53-78	79-105[f]	Total
0	0/50	3/50	9/47	7/38	19/50 (38)
30	2/50	2/48	3/46	15/43	22/50 (44)
60	2/50	2/48	6/46	16/40	26/50 (52)

[+]Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f] Final sacrifice at weeks 104-105.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Neoplastic Lesions- Rat Study

There were no statistically significant tumor findings in male or female rats (no p values < 0.05). The statistical analyses of the liver cholangiocarcinomas in the male rats were based upon Exact test for trend and the Fisher's Exact Test (Table 2).

Table 2. Furfural – F344/N Rat Study Male Liver Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Tumor Type	Dose (mg/kg/day)			Historical Control (HC)
	0	30	60	
Cholangiocarcinomas (%)	0/47 (0)	0/46 (0)	2[a]/46 (4)	SRI: 2/449 (0.4%) SD 0.88%
p =	0.1079	1.0000	0.2419	Overall: 3/2,145 (0.1%) SD= 0.52%

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First cholangiocarcinoma observed at week 81, dose 60 mg/kg/day.
Historical Incidence of Bile Duct Neoplasms in Male F344/N Rats at Southern Research Institute (SRI): 2/449 (0.4%); SD = 0.88%, Overall Historical Incidence: 3/2,145 (0.1%); SD= 0.52% (NTP, 1990)
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

D. Non-Neoplastic Lesions- Rat Study

Mild liver toxicity in the form of centrilobular necrosis and bile duct dysplasia with fibrosis were observed in male rats administered furfural (Table 3). Bile duct dysplasia with fibrosis is considered to be an early stage in the development of cholangiocarcinomas. Hematology was not performed and no other clinical signs were reported.

Table 3. Furfural – F344/N Rat Study Male Non-Neoplastic Lesions in the Liver (2-year)

	Dose (mg/kg/day)		
	0	**30**	**60**
Survivors to Terminal Sacrifice	31	28	24
Liver: Centrilobular necrosis	2	8	12
Liver: Bile Duct Dysplasia with Fibrosis	0	0	2

E. Adequacy of Dosing for Assessment of Carcinogenicity

The dose levels for this study were selected based on the results of a 16-day and 13-week studies. As shown in Table 4, mortality occurred at 90 mg/kg/day and 180 mg/kg/day. Because of treatment-related mortality, 60 mg/kg/day was selected as the high dose for the bioassay. The CARC determined that the doses tested were adequate, but not excessive, in both sexes to assess the carcinogenic potential of furfural. This was based on the presence of non-neoplastic and neoplastic lesions in male rats. No changes in body weight were observed throughout the 2-year study. The NTP tested only two doses as opposed to the guideline requirement (Part 158 Test Guideline study 870.4200) of three treatment levels and a control group. The CARC determined that this study is adequate since: the doses were selected based on the findings of the 16-day and 13-week studies. Non-neoplastic lesions and evidence for carcinogenicity were seen at the high dose.

Table 4. Mortality data for male and female F344/N rats in the 13-week oral gavage study of furfural.

	Dose (mg/kg/day)					
	0	**11**	**22**	**45**	**90**	**180**
Males	1/10[a]	0/10	0/10	0/10	1/10[a]	9/10[b]
Females	1/10[a]	0/10	0/10	0/10	4/10[b]	10/10[c]

[a]Death gavage related
[b]3 deaths were gavage related
[c] 1 death was gavage related

F. NTP Conclusions

The NTP concluded that there was some evidence of carcinogenic activity in male F344/N rats and no evidence of carcinogenic activity for female F344/N rats.

2. Carcinogenicity Study in Mice with Furfural

A. Experimental Design- Mouse Study

Furfural was administered in corn oil to B6C3F1 mice (50/sex/dose) at dose levels of 0, 50, 100, or 175 mg/kg bw/day, 5 days/wk via oral gavage for 2 years (104 weeks).

B. Discussion of Survival Data- Mouse Study

There were no statistically significant trends for survival for male or female mice (Tables 5 and 6), but there was a survival disparity with a statistically significant pair-wise comparison of the 100 mg/kg/day mid-dose group with the controls for male mice. However, no statistically significant pair-wise comparison for survival for male mice was seen at the high dose (175 mg/kg/day).

Table 5. Furfural – B6C3F$_1$ Mouse Study Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	Weeks				
	1-26	27-52	53-78	79-105[f]	Total
0	2/50	0/48	5/48	8/43	15/50 (30)
50	2/50	2/48	4/46	14/42	22/50 (44)
100	1/50	0/49	5/49	20/44	26/50 (52)[*]
175	5/50	1/45	4/44	13/40	23/50 (46)

[+]Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at weeks 104-105.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

Table 6. Furfural – B6C3F1 Mouse Study Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	Weeks				
	1-26	27-52	53-78	79-105[f]	Total
0	1/50	0/49	6/49	10/43	17/50 (34)
50	0/50	2/50	9/48	11/39	22/50 (44)
100	0/50	1/50	9/49	11/40	21/50 (42)
175	0/50	1/50	5/49	12/44	18/50 (36)

[+]Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at weeks 104-105.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

C. Neoplastic Lesions- Mouse Study

Male mice had statistically significant trends, and statistically significant pair-wise comparisons of the high dose with the controls, for liver adenomas, carcinomas, and adenomas and carcinomas combined, all at $p < 0.01$. The increase in carcinomas was particularly noteworthy, with the incidence in the high dose group being three-fold greater than the incidence in the vehicle control group. The kidney tumor findings in the male rat were not statistically significant. The statistical analyses of the tumors in the male mice were based upon the Exact test for trend and the Fisher's Exact Test (Tables 7 and 8).

Female mice had significant trends for liver adenomas, liver adenomas and carcinomas combined, and forestomach squamous cell papillomas, all at $p < 0.01$. There was also a significant pair-wise comparison of the 175 mg/kg/day dose group with the controls for liver adenomas at $p < 0.05$. The statistical analyses of the tumors in the male mice were based upon the Exact test for trend and the Fisher's Exact Test (Tables 9 and 10).

Table 7. Furfural – B6C3F$_1$ Mouse Study Male Hepatocellular Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Tumor Type	Dose (mg/kg/day)				
	0	50	100	175	Historical Control
Adenomas (%)	9/48 (19)	13[a]/46 (28)	11/48 (23)	19/44 (43)	SRI: 73/448 (16.3%) SD 9.51%
p =	0.00959**	0.19917	0.40105	0.00997**	Overall: 338/2,183 (15.5%) SD 7.01%
Carcinomas (%)	7/48 (15)	12[b]/46 (26)	6/48 (13)	21/44 (48)	SRI: 108/448 (24.1%), SD 7.16%
p =	0.00076**	0.12887	0.72385	0.00055**	Overall: 418/2,183 (19.1%), SD 7.42%
Combined (%)	16/48 (33)	22[c]/46 (48)	17/48 (35)	32[d]/44 (73)	SRI: 168/448 (34.5%) SD 9.57% Overall: 713/2,183 (32.7%), SD 8.55%
p =	0.00038**	0.11096	0.50000	0.00015**	

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First adenoma observed at week 70, dose 50 mg/kg/day.
[b]First carcinoma observed at week 62, dose 50 mg/kg/day.
[c]Three animals in the 50 mg/kg/day dose group had both an adenoma and a carcinoma.
[d]Eight animals in the 175 mg/kg/day dose group had both an adenoma and a carcinoma.
Historical incidence of hepatocellular neoplasms in Male B6C3F1 mice at Southern Research Institute (SRI) Adenoma Total: 73/448 (16.3%), SD 9.51% ; Overall: 338/2,183 (15.5%), SD 7.01%; Carcinoma Total: 108/448 (24.1%) , SD 7.16%; Overall:418/2,183 (19.1%), SD 7.42%; Adenoma or Carcinoma Total: 168/448 (37.5%), SD 9.57%; Overall 713/2,183 (32.7%), SD 8.55%. (NTP, 1990)
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 8. Furfural – B6C3F$_1$ Mouse Study Male Renal Cortical Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Tumor Type	Dose (mg/kg/day)				Historical Controls
	0	50	100	175	
Adenomas (%) p =	0/48 (0) 0.1777	0/46 (0) 1.0000	1[a]/48 (2) 0.5000	1[a]/44 (2) 0.4783	—
Carcinomas (%) p =	0/48 (0) 0.5054	1[b]/46 (2) 0.4894	0/48 (0) 1.0000	0/44 (0) 1.0000	—
Combined (%) p =	0/48 (0) 0.2790	1/46 (2) 0.4894	1/48 (2) 0.5000	1/44 (2) 0.4783	SRI: 0/448 Overall: 8/2,183

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First adenoma observed at week 104 simultaneously in final sacrifice animals at doses 100 and 175 mg/kg/day.
[b]First carcinoma observed at week 105 in a final sacrifice animal, dose 50 mg/kg/day.
Historical Incidence of renal cortical neoplasm in male B6C3F1 mice at Southern Research Institute (SRI):0/448 SD –not reported; Overall: 8/2,183 SD – not reported. (NTP, 1990)
Note: There were no statistically significant tumor findings in the kidney.

Table 9. Furfural – B6C3F1 Mouse Study Female Hepatocellular Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Tumor Type	Dose (mg/kg/day)				Historical Control
	0	50	100	175	
Adenomas (%) p =	1/49 (2) 0.0056**	3/48 (6) 0.3008	5[a]/49 (10) 0.1021	8/49 (16) 0.0154*	SRI 17/450 (3.8%) SD 2.73% Overall 104/2,188 (4.8%) SD 3.96%
Carcinomas (%) p =	4/49 (8) 0.3528	0/48 (0) 1.0000	2/49 (4) 0.8980	4[b]/49 (8) 0.6426	SRI 11/450 (2.4%) SD 2.40% Overall 60/2,188 (2.7%) SD 2.41%
Combined (%) p =	5/49 (10) 0.0095**	3/48 (6) 0.8592	7/49 (14) 0.3796	12/49 (24) 0.0538	SRI 28/450 (6.2%) SD 2.33% Overall 162/2,188 (7.4%) SD 4.98%

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First adenoma observed at week 70; [b]First carcinoma observed atweek 78.
Historical incidence of hepatocellular neoplasms in female B6C3F1 mice at Southern Research Institute (SRI). Adenoma Total: 17/450 (3.8%), SD 2.73%; Overall: 104/2,188 (4.8%) , SD 3.96%; Carcinoma Total: 11/450 (2.4%), SD 2.40%; Overall: 60/2,188 (2.7%), SD 2.41 %; Adenoma or Carcinoma Total: 28/450 (6.2%), SD 2.33%; Overall: 162/2,188 (7.4%), SD 4.98%. (NTP, 1990)
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 10. Furfural – B6C3F1 Mouse Study- Female Forestomach Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Tumor Type	Dose (mg/kg/day)				Historical Control
	0	50	100	175	
Papillomas (%)	1/49 (2)	0/48 (0)	1[a]/49 (2)	6/49 (12)	SRI: 8/446 (1.8%) SD 2.73% Overall: 34/2,144 (1.6%) SD 2.74%
p =	0.00328**	1.00000	0.75258	0.05574	

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First adenoma observed at week 75, dose 100 mg/kg/day.
Historical incidence of forestomach squamous cell neoplasm in female B6C3F1 mice at Southern Research Institute (SRI) – Papilloma Total: 8/446 (1.8%), SD 2.73%; Overall: 34/2,144 (1.6%), SD 2.74%; Papilloma or Carcinoma Total: 8/446 (1.8%), SD 2.73%,; Overall: 37/2,144 (1.7%), SD 2.74%.(NTP, 1990)
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

D. Non-Neoplastic Lesions- Mouse Study

Liver toxicity, in the form of chronic inflammation and increased hepatic pigmentation in male and female mice, was observed in the 2-year study (Table 11).

Table 11. Incidence data of non-neoplastic lesions in B6C3F1 mice in the 2-year oral gavage study of furfural.

	Dose (mg/kg/day)			
	0	**50**	**100**	**175**
Lesions Type	**Males**			
Liver Pigmentation	0/50	0/50	8/49	18/50
Chronic Inflammation	0/50	0/50	8/49	18/50
	Females			
Liver Pigmentation	0/50	0/50	0/50	11/50
Chronic Inflammation	0/50	0/50	1/50	8/50

E. Adequacy of Dosing for Assessment of Carcinogenicity

The dose levels for this study were selected based on the results of a 13-week study. As shown in Table 12, mortality occurred at the 600 and 1200 mg/kg/day dose levels. As shown in Table 13, liver lesions were also seen at these doses, as well as at 300 mg/kg/day. Based on these findings, 175 mg/kg/day was selected as the highest dose for the carcinogenicity study. The CARC considered the doses tested adequate, but not excessive, in both sexes to assess the carcinogenic potential of furfural. This was based on the presence of non-neoplastic lesions in the liver.

Table 12. Mortality data for B6C3F1 mice in the 13-week oral gavage study of furfural

	Dose (mg/kg/day)					
	0	75	150	300	600	1200
Males	0/10	0/10	0/10	0/10	9/10	10/10
Females	1/10[d]	0/10	9110[d]	0/10	9/10	10/10

[d] Death was gavage related

Table 13. Incidence data of non-neoplastic lesions for B6C3F1 mice in the 13-week oral gavage study of furfural.

	Dose (mg/kg/day)					
	0	75	150	300	600	1200
	Males					
Centrilobular Coagulative Necrosis	0/10	0/10	1/10	1/10	9/10	8/10
	Females					
Centrilobular Coagulative Necrosis	0/10[d]	0/10	0/10[d]	0/10	/10	2/10

F. NTP Conclusions

NTP concluded that there was clear evidence of carcinogenic activity in male $B6C3F_1$ mice and some evidence of carcinogenic activity in female $B6C3F_1$ mice.

Furfuryl Alcohol

Reference: NTP (National Toxicology Program). 1999. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Furfuryl Alcohol (CAS No. 98-00-0) in F344/N rats and B6C3F1 mice (Inhalation Studies). NTP-TR-482, MRID 49161601.

A carcinogenicity inhalation study in F344/N rats was conducted by the NTP. In this study, F344/N male and female rats (50/sex/dose) were treated via inhalation with 0, 2, 8, or 32 ppm for 6 hours/day, 5 days/wk for 2 years (105 weeks). Rats were approximately 6 weeks of age at the start of the study.

Survival data and tumors were evaluated by the Office of Pesticide Programs Health Effects Division the results of which are presented below. For further details, see TXR 0056739, Memorandum from L. Brunsman to A. Howard (September 3, 2013).

3. Carcinogenicity Study in Rats - Furfuryl Alcohol

A. Experimental Design- Rat Study

Furfuryl alcohol was administered via inhalation to F344/N rats (50/sex/concentration) at concentrations of 0, 2, 8, or 32 ppm, 5 days/wk for 2 years (104 weeks) in a carcinogenicity study.

B. Discussion of Survival and Tumor Data

There were statistically significant survival disparities among the dose groups for male rats, as no male rats in the high dose group survived to termination (Table 14). However, the majority of the deaths in males at 32 ppm occurred after study week 91. Survival of all other exposed groups of male rats was similar to that of the chamber control group. Although the cause of death for the males at the high dose was not reported, nephropathy, a common spontaneous renal disease of F344 rats, was increased in males at 32 ppm (severity level, 3.7) compared to chamber controls (2.9). Additionally, males exposed to 32 ppm also exhibited an increased incidence of lesions related to kidney failure including parathyroid gland hyperplasia (39/50 vs. 9/49 in the controls ; $P<0.01$) and fibrous osteodystrophy (34/50 vs. 2/50 <p.0.01). There were no significant survival disparities for female rats (Tables 15).

Table 14. Furfuryl Alcohol – F344/N Rat Study Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks				
	1-26	27-52	53-78	79-105[f]	Total
0	1/50	3/49	8/46	30/38	42/50 (84) **
2	0/50	0/50	10/50	35/40	45/50 (90)
8	0/50	0/50	11/50	30/39	41/50 (82)
32	0/50	1/50	9/49	40/40	50/50 (100) **

[+]Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 105.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

Table 15. Furfuryl Alcohol – F344/N Rat Study Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks				
	1-26	27-52	53-78	79-105[f]	Total
0	1/50	0/49	4/49	19/45	24/50 (48)*
2	0/50	0/50	6/50	18/44	24/50 (48)
8	0/49	0/49	5/49	22/44	27/49 (55)
32	0/50	1/50	8/49	25/41	34/50 (68)*

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 105.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

C. Neoplastic Lesions- Rat Study

Male rats had a significant trend for nasal epithelial squamous cell carcinomas at $p < 0.05$. There was also a significant trend at $p < 0.01$, and a significant pair-wise comparison of the high dose group with the controls at $p < 0.05$, for combined nasal lateral wall adenomas, epithelial adenomas, epithelial carcinomas and epithelial squamous cell carcinomas in male rats. The statistical analyses of the nasal lateral wall and nasal epithelial tumors in the male rats were based upon Peto's prevalence test (Table 16).

Female rats had a significant trend for nasal epithelial adenomas at $p < 0.05$. There were no statistically significant pair-wise comparisons of the dosed groups with the controls. Ad hoc analyses were run on the female rat kidney tumors because there was no individual animal data provided for the step sectioning performed on this tissue. The statistical analyses of the nasal and kidney tumors in female rats were based upon the Peto's prevalence test (Tables 17 and 18).

Table 16. Furfuryl Alcohol – F344/N Rat Study Male Nasal Tumor Rates[+] and Peto's Prevalence Test Results

Tumor Type	Dose (ppm)				Historical Control (NTP, 1999)
	0	2	8	32	
Lateral wall Adenomas (%) p =	0/9 (0) 0.61596	1[a]/8 (13) 0.28185	0/10 (0) NA	0/0 (0) NA	
Epithelial Adenomas (%) p =	0/34 (0) 0.60688	0/34 (0) NA	1[b]/34 (3) 0.15391	0/33 (0) NA	SRI: 1/897 (0.1%) SD 0.5%
Epithelial Carcinoma (%) p =	0/30 (0) 0.06097	0/32 (0) NA	0/30 (0) NA	1[c]/28 (4) 0.18770	0/897
Epithelial Squamous Cell Carcinomas (%) p =	0/30 (0) 0.01085*	0/32 (0) NA	0/30 (0) NA	3[d]/28 (11) 0.09177	0/897
Combined (%) p =	0/34 (0) 0.00643**	1/34 (3) 0.28185	1/34 (3) 0.15866	4/33 (12) 0.03682*	—

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First Lateral wall Adenoma observed at week 103, dose 2 ppm
[b]First Epithelial Adenoma observed at week 84, dose 8 ppm
[c]First Epithelial Carcinoma observed at week 87, dose 32 ppm

[d]First Epithelial Squamous Cell Carcinoma observed at week 87, dose 32 ppm
Historical incidence of nasal neoplasms in chamber control male F344/N rats at Southern Research Institute (SRI) – Adenoma Total: 1/897 (0.1%), SD 0.5%; Carcinoma: 0/897; Squamous Cell Carcinoma: 0/897.(NTP, 1999)
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 17. Furfuryl Alcohol – F344/N Rat Study Female Nasal Tumor Rates[+] and Peto's Prevalence Test Results

Tumor Type	Dose (ppm)				Historical Control (NTP, 1999)
	0	2	8	32	
Lateral Wall Adenomas	0/44	0/45	1[a]/46	0/41	Overall Total: 1/892 (0.1%) SD 0.5%
(%)	(0)	(0)	(2)	(0)	
p =	0.61881	NA	0.19324	NA	
Epithelial Adenomas	0/26	0/26	0/22	1[b]/16	
(%)	(0)	(0)	(0)	(6)	
p =	0.01897*	NA	NA	0.10120	
Combined	0/44	0/45	1/46	1/41	—
(%)	(0)	(0)	(2)	(2)	
p =	0.12813	NA	0.19324	0.10120	

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First Lateral Wall Adenoma observed at week 78, dose 8 ppm
[b]First Epithelium Adenoma observed at final sacrifice week 105, dose 32 ppm
Historical incidence of nasal adenoma in chamber control female F344/N rats at Battelle Pacific Northwest Labs — Overall Total- 1/892 (0.1%) SD 0.5% (NTP, 1999).
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 18. Furfuryl Alcohol – F344/N Rat Study Female Renal Tubule Tumor Rates[+] and *ad hoc* Fisher's Exact Test and Exact Trend Test Results

Tumor Type	Dose (ppm)				Historical Control (NTP, 1999)
	0	2	8	32	
Adenomas (%) p =	0/50 (0) 0.0736	0/49 (0) 1.0000	2/49 (4) 0.2424	2/50 (4) 0.2475	Overall 1/898 (0.1%) SD 0.5%
Carcinoma (%) p =	0/50 (0) 0.5000	1/49 (2) 0.4950	0/49 (0) 1.0000	0/50 (0) 1.0000	Overall 4/898 (0.5%) SD 0.9%
Combined (%) p =	0/50 (0) 0.1421	1/49 (2) 0.4950	2/49 (4) 0.2424	2/50 (4) 0.2475	Overall 5/898 (0.6%) SD 0.9%

[+]Number of tumor bearing animals/Number of animals examined.
Historical incidence of renal tubule neoplasms in chamber control female F344/N rats at Battelle Pacific Northwest Labs – Adenoma Overall Total -1/898 (0.1%) SD 0.5%; Carcinoma – 4/898 (0.5%) SD 0.9%; Adenoma and Carcinoma – 5/898 (0.6%) SD 0.9% (NTP, 1999).
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

D. Non-Neoplastic Lesions- Rat Study

The primary target organs of the test substance for non-neoplastic effects were the nose and the kidney. The non-neoplastic microscopic lesions observed in the nose of male and female rats are summarized in Table 19. Furfuryl alcohol exposed groups had increased incidences of non-neoplastic lesions in the nose relative to those in controls. The incidences of hyperplasia of the lateral wall, atrophy and metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium were statistically elevated relative to controls at all three exposure concentrations in both sexes (typically $p \leq 0.01$). The incidences of nasal lesions increased with increasing exposure concentration, the most marked increase occurring between 2 and 8 ppm in both sexes. All animals exposed at 32 ppm were affected. The severity also increased with increasing concentration to a similar extent in both males and females. Necrosis was not observed in either sex, demonstrating that the basic structure of the nasal turbinates and mucosal lining remained intact. However, somewhat greater toxicity was evident in males with higher incidence rates. For example, fibrosis of the olfactory epithelium in males occurred in 1/50, 26/50, and 40/50 at 2, 8, and 32 ppm, respectively, whereas the incidences in females were 0/50, 16/50, and 31/50. The incidences of hyperplasia and squamous metaplasia, adaptive responses to chronic irritation, when not similar in males and females, were higher in males. The squamous metaplasia of the respiratory epithelium in males was 3/50 and 26/50 at 8 and 32 ppm, while the respective incidences in females were 2/50 and 10/50.

TABLE 19: Incidences of selected non-neoplastic lesions of the nose in rats exposed for 2 years to furfuryl alcohol.

	Dose (ppm)			
	0	2	8	32
Males -Number Examined	**50**	**50**	**50**	**50**
Suppurative inflammation[a]	3 (1.0)[b]	6 (1.5)	17** (1.7)	44** (2.1)
Glands, hyperplasia	0	0	22** (1.0)	49** (2.3)
Lateral wall, hyperplasia	1 (1.0)	49** (1.5)	50** (2.4)	50** (3.5)
Lateral wall, squamous metaplasia	1 (1.0)	1 (1.0)	8* (1.1)	33** (1.3)
Olfactory epithelium, atrophy	1 (1.0)	12** (1.1)	47** (1.8)	50** (2.4)
Olfactory epithelium, hyaline degeneration	42 (1.3)	48 (1.5)	50** (2.6)	47 (2.7)
Olfactory epithelium, fibrosis	0	1 (1.0)	26** (1.0)	40** (2.0)
Olfactory epithelium, hyperplasia	0	1 (1.0)	42** (1.0)	40** (1.8)
Olfactory epithelium, metaplasia	1 (1.0)	8* (1.3)	37** (1.5)	49** (2.2)
Respiratory epithelium, hyaline degeneration	12 (1.0)	14 (1.0)	45** (1.6)	3* (1.7)
Respiratory epithelium, hyperplasia	0	26** (1.8)	50** (2.5)	50** (3.5)
Respiratory epithelium, squamous metaplasia	0	0	3 (1.0)	26** (1.4)
Females -Number Examined	**49**	**50**	**48**	**49**
Suppurative inflammation[a]	4 (2.3)	1 (2.0)	5 (1.4)	23** (1.7)
Glands, hyperplasia	0	0	24** (1.0)	46** (2.2)
Lateral wall, hyperplasia	0	39** (1.3)	48** (2.1)	49** (3.3)
Lateral wall, squamous metaplasia	0	1 (1.0)	0	24** (1.0)
Olfactory epithelium, atrophy	0	6* (1.3)	44** (1.7)	49** (2.3)
Olfactory epithelium, hyaline degeneration	43 (1.2)	50* (1.6)	47 (2.7)	48 (3.3)
Olfactory epithelium, fibrosis	0	0	16** (1.3)	31** (1.7)
Olfactory epithelium, hyperplasia	0	0	31** (1.2)	41** (1.5)
Olfactory epithelium, metaplasia	0	5* (1.2)	37** (1.5)	48** (2.2)
Respiratory epithelium, hyaline degeneration	23 (1.0)	39** (1.2)	45** (1.9)	6** (2.0)
Respiratory epithelium, hyperplasia	0	18** (1.4)	40** (2.1)	49** (3.2)
Respiratory epithelium, squamous metaplasia	0	0	2 (1.0)	10** (1.2)

*Significantly different ($p<0.05$) from chamber control group by Poly-3 test ** $p\leq 0.01$
[a]Number of animals with lesions
[b]Average severity grade of lesion in (); 1=minimal, 2=mild, 3=moderate, 4=marked

E. Adequacy of Dosing for Assessment of Carcinogenicity

The concentrations tested were selected based on the results of a 14-week inhalation study. Incidences and severities of cytological alterations in the nasal mucosa were associated with exposure to furfuryl alcohol at concentrations of 8 ppm or greater. Based on the histopathological lesions of the nasal mucosa, 32 ppm was selected as the high concentration for the bioassay. The CARC determined that the concentrations tested in the study were adequate in both sexes to assess the carcinogenic potential of furfuryl alcohol. This was based on the presence of non-neoplastic and neoplastic lesions of the nasal cavity. Mean body weights of 32 ppm males were less than those of the chamber controls beginning at week 19 (94% of control values); by week 91, the body weights of 32 ppm males were 76% of those of the chamber controls. Mean body weights of 2 and 8 ppm male groups and all exposed female groups were similar to those of the chamber controls, throughout the study. Although there was 100% mortality at the high dose, the CARC did not consider this concentration to be excessive, since survival was 80% at 18 months and 32 % at week 91 which met the test guideline requirement (i.e., survival no less than 50% at 18 months for rats). Additionally all rats were available for histopathological examination (i.e., no more than 10 percent of any group was lost due to autolysis, cannibalism, or management problems, as required by the test guideline).

F. NTP Conclusions

The NTP concluded that there was some evidence of carcinogenic activity of furfuryl alcohol in male rats and equivocal evidence of carcinogenic activity of furfuryl alcohol in female rats.

4. Carcinogenicity Study in Mice - Furfuryl Alcohol

A. Experimental Design- Mouse Study

Furfuryl alcohol was administered via inhalation to B6C3F1 mice (50/sex/dose) at concentrations of 0, 2, 8, or 32 ppm, 5 days/wk for 2 years (104 weeks) in a combined carcinogenicity study.

B. Discussion of Survival Data- Mouse Study

There were no statistically significant survival disparities among the dose groups in male or female mice (Table 20 and Table 21, respectively).

Table 20. Furfuryl Alcohol – B6C3F$_1$ Mouse Study Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks				
	1-26	27-52	53-78	79-105	Total
0	0/50	0/50	6/50	10/44	16/50 (32)
2	0/50	2/50	2/48	10/46	14/50 (28)
8	0/50	2/50	9/48	9/39	20/50 (40)
32	0/50	0/50	2/50	10/48	12/50 (24)

[+]Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 105.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

Table 21. Furfuryl Alcohol – B6C3F$_1$ Mouse Study Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks				
	1-26	27-52	53-78	79-105	Total
0	0/50	1/50	3/49	12/46	16/50 (32)
2	0/49[a]	1/49	5/48	9/42[b]	15/48 (31)
8	0/50	0/50	3/50	14/46[b]	17/49(35)
32	0/50	0/50	0/50	10/50	10/50 (20)

[+]Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 105.
[a]One female in the 2 ppm dose group was found to be pregnant and removed from the study.

[b]Two accidental deaths, both at week 98, one each in the 2 and 8 ppm dose groups.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Neoplastic Lesions- Mouse Study

Male mice had significant trends for renal tubule adenomas at $p < 0.05$. There was also a significant trend at $p < 0.01$, and a significant pair-wise comparison of the 32 ppm dose group with the controls at $p < 0.05$, for renal tubule adenomas and/or carcinomas combined. The statistical analyses of the kidney tumors in male mice were based upon the ad hoc Exact test for trend and the Fisher's Exact Test (Table 22). Ad hoc analyses were run on the male mouse kidney tumors because there was no individual animal data provided for the step sectioning performed on this tissue. There was no evidence of carcinogenic activity with furfuryl alcohol in female mice.

Table 22. Furfuryl Alcohol – B6C3F$_1$ Mouse StudyMale Renal Tubule Tumor Rates[+] and *ad hoc* Fisher's Exact Test and Exact Trend Test Results

Tumor Type	Dose (ppm)				Historical Control (NTP, 1999)
	0	2	8	32	
Adenomas (%) p =	0/50 (0) 0.01538*	0/49 (0) 1.00000	0/49 (0) 1.00000	3/50 (6) 0.12121	Overall: 3/1,093 (0.3%) SD 0.6%
Carcinomas (%) p =	0/50 (0) 0.06281	0/49 (0) 1.00000	0/49 (0) 1.00000	2/50 (4) 0.24747	Overall: 1/1,093 (0.1%) SD 0.4%
Combined (%) p =	0/50 (0) 0.00088**	0/49 (0) 1.00000	0/49 (0) 1.00000	5/50 (10) 0.02814*	Overall: 4/1,093 (0.4%) SD 1.0%

[+]Number of tumor bearing animals/Number of animals examined.
Historical incidence of renal tubule neoplasms in chamber control male B6C3F1mice at Battelle Pacific Northwest Labs – Adenoma Overall Total -3/1,093 (0.3%) SD 0.6%; Carcinoma – 1/1,093 (0.1%) SD 0.4%; Adenoma and Carcinoma – 4/1,093 (0.4%) SD 1.0% (NTP, 1999).
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

D. Non-Neoplastic Lesions- Mouse Study

The primary target organs of the test substance for non-neoplastic effects in mice were the nose, the kidney, and the eye (female mice only). In the nose, there were a variety of non-neoplastic histological changes which were statistically significantly increased in the treated groups, vs. chamber controls. The non-neoplastic lesions observed in the nose of male and female mice are summarized in Table 23. The severity of the lesions increased with increasing dose in both males and females and 3/50 females and 1/50 males at 32 ppm had necrosis of the respiratory epithelium. The change was not statistically significant, but does suggest that there is an increase in severity with increasing dose.

In the kidney, nephropathy was observed in all groups of male and female mice (summarized in Table 24). The severity increased with increasing dose in male mice only. The nephropathy consisted of necrosis and regeneration of the renal tubule epithelium and inflammation and fibrosis in the interstitium. The incidence of renal tubule degeneration in male mice exposed to 32 ppm was greater (statistically significantly) than in the controls. Additionally, renal tubule degeneration was observed and consisted of slightly distended tubules with lumens containing eosinophilic, finely granular material. Some degenerate tubules contained one to a few enlarged epithelial cells with large, sometimes pleomorphic nuclei.

There was a high incidence of degeneration of the cornea in female mice only, at 32 ppm (3/49, 1/49, 4/49, 26/50; at 0, 2, 8, and 32 ppm, respectively). Corneal degeneration consisted of mineralization of the stroma beneath the corneal epithelium.

Table 23. Incidences of selected non-neoplastic lesions of the nose in mice exposed for 2 years to furfuryl alcohol.

	Dose (ppm)			
	0	2	8	32
Males -Number Examined	**50**	**49**	**49**	**50**
Suppurative inflammation[a]	7 (1.4)[b]	11 (1.2)	27** (1.3)	28** (1.7)
Glands, hyperplasia	0	10** (1.0)	48** (1.8)	46** (3.3)
Glands, squamous metaplasia	0	6* (1.0)	35** (1.1)	47** (1.5)
Lateral wall, squamous metaplasia	0	9** (1.0)	10** (1.7)	20** (1.5)
Olfactory epithelium, atrophy	3 (1.0)	15** (1.2)	49** (2.2)	50** (3.6)
Olfactory epithelium, hyaline degeneration	2 (1.5)	3 (1.7)	21** (1.3)	39** (2.0)
Olfactory epithelium, metaplasia	0	12** (1.1)	49** (2.1)	50** (3.5)
Respiratory epithelium, hyaline degeneration	5 (1.0)	18** (1.1)	42** (1.3)	45** (1.2)
Respiratory epithelium, squamous metaplasia	0	2 (1.0)	10** (1.1)	20** (1.4)

	Dose (ppm)			
	0	2	8	32
Respiratory epithelium, necrosis	1 (2.0)	0	0	1 (1.0)
Respiratory epithelium, regeneration	0	1 (1.0)	13** (1.0)	21** (1.0)
Females -Number Examined	**50**	**48**	**49**	**50**
Suppurative inflammation	5 (1.2)	12* (1.1)	25** (1.5)	42** (2.0)
Glands, hyperplasia	0	33** (1.1)	46** (2.8)	47** (3.1)
Glands, squamous metaplasia	1 (1.0)	1 (1.0)	34** (1.1)	46** (1.5)
Lateral wall, squamous metaplasia	3 (1.0)	14** (1.4)	16** (1.4)	36** (1.9)
Olfactory epithelium, atrophy	2 (1.0)	35** (1.2)	49** (3.0)	50** (3.6)
Olfactory epithelium, hyaline degeneration	7 (1.3)	14 (1.4)	28** (1.8)	45** (2.2)
Olfactory epithelium, metaplasia	0	31** (1.2)	49** (3.0)	49** (3.6)
Respiratory epithelium, hyaline degeneration	19 (1.4)	44** (1.5)	49** (1.3)	48** (1.4)
Respiratory epithelium, squamous metaplasia	1 (1.0)	9** (1.8)	21** (1.7)	39** (1.9)
Respiratory epithelium, necrosis	0	0	2 (2.5)	3 (1.3)
Respiratory epithelium, regeneration	0	0	9** (1.0)	13** (1.2)

*Significantly different ($p<0.05$) from chamber control group by Poly-3 test

** $p\leq 0.01$

[a]Number of animals with lesions

[b]Average severity grade of lesion in (); 1=minimal, 2=mild, 3=moderate, 4=marked

Table 24. Incidences of Non-neoplastic Lesions of the Kidney in B6C3F1 mice exposed for 2 years to furfuryl alcohol. .

	Dose (ppm)			
	0	2	8	32
Males -Number Examined	**50**	**49**	**49**	**50**
Nephropathy	49 (1.2)	48 (1.4)	43 (1.5)	47 (1.8)
Renal Tubule, Degeneration	0	0	1 (1.0)	48**(1.0)
Renal Tubule, Hyperplasia	1 (1.0)	3 (1.0)	0	3 (1.7)
Females -Number Examined	**50**	**48**	**49**	**49**
Nephropathy	41 (1.0)	35 (1.1)	40 (1.2)	39 (1.0)

** $p\leq 0.01$; Average severity grade of lesion in (); 1=minimal, 2=mild, 3=moderate, 4=marked

E. Adequacy of Dosing for Assessment of Carcinogenicity

Exposure to concentrations of furfuryl alcohol (via inhalation) at up to 32 ppm for 14 weeks, produced few overt signs of toxicity in mice. No effects were observed on body weight in male or female mice. The most significant response occurred in the nose where a spectrum of inflammatory, degenerative, and proliferative lesions of the respiratory and olfactory epithelium were observed following exposure to furfuryl alcohol vapor. In mice, nasal lesions of the olfactory and respiratory epithelium were observed in all exposed groups. Changes were more severe in the anterior portion than in the posterior portion of the nose and severity increased with increasing exposure concentration. The integrity of the nasal mucosa or nasal passages was not disrupted, however. Therefore, for the cancer study, the concentrations of 2, 8, and 32 ppm were selected. The CARC determined that the highest concentration tested (32 ppm) was considered to be adequate, but not excessive, in both sexes to assess the carcinogenic potential of furfuryl alcohol. This was based on the presence of non-neoplastic and neoplastic lesions in the kidneys. Mean body weights of exposed males were generally similar to those of the chamber control group throughout the study. Mean body weights of females exposed to 2, 8, or 32 ppm were less than those of the chamber control group beginning at weeks 59, 59, or 39, respectively. Hematology was not performed.

F. NTP Conclusions

The NTP stated that there was some evidence of carcinogenic activity of furfuryl alcohol in male mice and no evidence of carcinogenic activity of furfuryl alcohol in female mice.

IV. TOXICOLOGY

1. Metabolism

A. Mammalian Metabolism of Furfural

No guideline metabolism studies are available for furfural. However, a study was identified in the open scientific literature that is considered acceptable for regulatory use. In this study, furfural was administered via gavage (in corn oil) and was rapidly absorbed and excreted, with about 80% of the elimination occurring within 24 hours. The major route of excretion was in the urine, in which 85% (out of a total recovered dose of 90%) was found by 72 hours. There were no changes in excretion indicative of saturation of excretion with increasing dose. Expired radioactivity (as carbon dioxide) was a minor route of excretion at 6.6% and was measured for the high dose only. The feces were also a minor route of excretion at ≤2% of the administered dose. Furfural was retained in tissues at low levels of less than 1% of the administered dose (range 0.1 ± 0.1% at 0.127 mg/kg to 0.6 ± 0.1% at 12.5 mg/kg), indicating low potential for bioaccumulation.

Furoylglycine was the major urinary metabolite for both the high and low dose groups, comprising over 75% of urinary metabolites by 48 hours. Furoic acid and furanacrylic acid were minor urinary metabolites that were present at <5% after 48 hours. The average levels of unidentified urinary metabolites were low, at less than 2%.

These results support a metabolic pathway in which furfural is converted to furanacrylic acid (presumably by condensation with acetyl-CoA), which is excreted in the urine (a minor pathway) or oxidized to furoic acid (the major pathway). Furoic acid can be excreted unchanged in the urine (a minor pathway), decarboxylated and exhaled as carbon dioxide (a minor pathway), or conjugated with glycine to form furoylglycine, which is excreted in urine (the major pathway).

B. Plant and Soil Metabolites of Furfural

Furfural degrades to 2-furoic acid and furfuryl alcohol in aerobic soil biodegradation environments. In anaerobic environments, ring opened metabolites propionic acid and acetic acid are also observed. Upon aqueous photolysis, several major ring opened products are formed; they include succinic acid, malonic acid, 2-ketoglutaric acid, propionic acid and formic acid. Some of these ring opened products are part of the Kreb's cycle (***e.g.*** 2-ketoglutaric acid and succinic acid). It appears that, in soils, furfural metabolites may be of higher exposure concern than the parent due to furfural's lability.

2. Mutagenicity

A. Furfural

Furfural has been studied in a comprehensive battery of well-done genetic toxicology assays, many of which were sponsored by the National Toxicology Program (NTP) and are summarized below:

In Vitro Studies

Gene Mutation

As part of the NTP genetic toxicology screening of 270 chemical, furfural and furfuryl alcohol were tested by independent laboratories in the *Salmonella typhimurium* mammalian microsome mutagenicity assay using the standard plate incorporation and preincubation assays (Mortelmans *et al*., 1986). Both test material were negative in *S. typhimurium* TA100, TA1535, TA1537 or TA98 up to cytotoxic concentrations (furfural: ≥ 3333 µg/plate-S9 or + 10% rat or hamster S9) or the highest dose tested (furfural alcohol: 10,000 µg/plate-S9 or + 10% rat or hamster S9).

Furfural was also tested in *S. typhimurium* TA100, TA1535, TA1537, TA98 or TA102 for reverse gene mutations (MRID 46011017) at concentrations up to the limit dose for this test system (5000 µg/plate). Results were negative with or without S9 activation.

In another *Salmonella* mutagenicity assay, Marnett *et al*. (1985) found that furfural was not mutagenic in a preincubation assay with *S. typhimurium* strains TA102 or TA104 up to the maximum noncytotoxic concentration (1 μmole). These strains were used because they are more sensitive indicators of aldehyde and ketone mutagenicity than the standard Ames tester strain battery.

In contrast to the uniformly negative bacterial gene mutation assays, McGregor *et al.* (1988) demonstrated that furfural in the absence of S9 activation (an S9-activated assay was not performed), induced increases in the mutation frequency (MF) of L5178Y tk^+/tk^- mouse lymphoma cells. The response was dose-related (ranging from 1.6-fold increase at 100 μg/mL to 11.3-fold at 400 μg/mL), occurred at moderately cytotoxic concentrations (65- 11% relative total growth, RTG, respectively) and was confirmed in a repeat assay (2.3-fold increase in the MF with 27% RTG at 200 μg/mL). It is of note that when the global evaluation factor (GEF), which is a more recently accepted approach to evaluate mouse lymphoma data and is recommended by the international workshop on genotoxicity (Moore *et al*., 2002), was applied to these data, the conclusion remained positive.

Chromosome Aberrations

NTP also sponsored an *in vitro* mammalian cell chromosome aberration assay with furfural. In this study, Stich *et al.* (1981), exposed Chinese hamster ovary (CHO) cells to 0, 200, 300, 400 or 500 μg/mL -S9 for 8-10 hours or 0, 500, 760, 1000, or 1230 μg/mL +S9 for 2 hours. Due to marked cell cycle delay, cultures were harvested at 22- 23.5 hours after treatment. Metaphase analysis revealed that in the absence, but not presence, of S9-activation, the percentage of cells with chromosome aberrations was pair-wise significantly ($p<0.05$) increased at 400 and 500 μg/mL-S9 with a significant ($p<0.001$) trend.

Other Mutagenic Mechanisms

As part of the comprehensive investigation of the toxicology of furfural, NTP sponsored an *in vitro* investigation of sister chromatid exchanges (SCEs) in CHO cells. Accordingly, Stich *et al*. (1981), exposed CHO cells to 0, 11.7, 38.9 or 117 μg/mL -S9 for 8-10 hours or 0, 117, 389, or 1170 μg/mL +S9 for 2 hours. Cells were processed and second division metaphases were analyzed. All non-activated or S9-activated concentrations induced significant pairwise ($p<0.01$) and dose-related increases (trend: $p<0.001$) in the percentage SCEs/chromosomes. The response was appreciably stronger in the absence of metabolic activation.

The ability of furfural and furfuryl alcohol to induce SCEs in mammalian cells was also assayed by Gomez-Arroyo and Souza (1985). In this study, human lymphocytes, collected from healthy donors, were dosed with 3.5-14.0 x 10^{-5} M furfural or 3.3-9.9 x 10^{-3} M furfuryl alcohol for 70 hours; 50 metaphases per duplicate culture were examined and SCE frequencies were determined. Results indicated that furfural at 7.0 and 14.0 x 10^{-5} M induced significant ($p<0.001$) and dose-related increases in SCEs. By contrast, furfuryl alcohol was negative. The effect of comparable concentrations of furfural on the mitotic spindle in the human lymphocytes from healthy donors was further evaluated. Data for the 24- and 48-hour harvest intervals show significant ($p<0.001$) and dose-related increases in c-mitosis, indicative of a mitotic poison along with significant ($p<0.05$-0.001) and dose-related increased mitotic indices, indicative of stimulated cell division. The percentage of tetraploid cells was also increased significantly ($p<0.05$) at 14 x 10^{-5} M furfural but only at the 48-hour cell harvest.

Additional blood samples were collected from 6 workers occupationally exposed to furfural or furfuryl alcohol. The analysis of SCE in these workers showed no significant differences compared to the control group of 6 workers without exposure.

In Vivo Studies

In a *Drosophila melangaster* sex -linked recessive lethal mutation assay, Woodruff *et a*l. (1985) exposed 24-hour old Canton-S males to furfural either by abdominal injection (100 ppm, 24-hour recovery) or feeding (1000 ppm, 3 days). Treated males were mated with three *Basc* females for 3 days and remated with fresh females to produce three broods which sampled sperm over the entire period of spermatogenesis. A significant ($p<0.05$) increase in sex-linked recessive lethal mutations was observed in the male germ cells after injection of 100 ppm furfural. No increases were seen in the feeding phase of study. In a follow-up experiment, the same investigators found that the administration of 100 ppm via injection did not induce reciprocal translocations in *D. melanogaster* males.

In the NTP *in vivo* mouse bone marrow cytogenetic assays, furfural was neither clastogenic nor induced SCEs in the bone marrow cells of male B6C3F1 mice administered doses of 0, 50, 100 or 200 mg/kg by intrapertoneal injection.

From these data it can be concluded that there is evidence of gene mutations *in vitro* in mammalian cells (mouse lymphoma L518Y) but not in bacteria. Similarly, there is convincing evidence of chromosome aberrations (CHO cells) and SCE induction (human lymphocytes and CHO cells) *in vitro* but this genotoxic activity is not expressed *in vivo* in mouse bone marrow cells. Sex-linked recessive lethal mutations in *D. melangaster* male germinal cells were also seen following abdominal injection but not when furfural was administered via feeding for 3 days. Based on the few studies reporting evidence of gene mutations, the European Union rapporteur of the risk assessment for furfural recommended that an *in vivo* gene mutation assay should be performed to elucidate the biological relevance of the genetic toxicology results.

Accordingly, an *in vivo* gene mutation assay with λlacZ-transgenic male mice (MRID 46011018) was submitted to the Agency. In this study, five groups of 15 male transgenic mice with *lacZ* genes as the mutational target received furfural prepared in corn oil at 0, 37.5, 75, 150 or 300 mg/kg/day by oral gavage for 28 days. On Day 28, 3 mice/group were sacrificed and selected tissues (*i.e.,* liver) were collected and examined histologically. On Days 62 and 63, the remaining animals were sacrificed and genomic DNA was harvested from the liver. The *lacZ* genes were packaged in lambda phages, mixed with *Escherichia coli* C *lacZrec*A*gal*E, plated and the mutation frequencies (MFs) were determined.

Dose selection was based on the findings of a 13-week subchronic toxicity study with a NOAEL of 75 mg/kg/day; 300 mg/kg/day was selected as the high dose which was expected to induce hepatotoxicity (Irwin, 1990). In the mutational assay, furfural was tested up to a toxic dose (300 mg/kg/day) based on three unscheduled deaths (3 of 10 animals), significant increases in both the absolute and relative liver weights accompanied by findings of slight centrilobular hypertrophy (3 of 3 mice), focal hemorrhage and inflammatory response (1of 3), and focal aggregates of mononuclear cells (1of 3) after 28 days of treatment. Despite the evidence of hepatotoxicity, there was no indication of a mutagenic response in the livers harvested from mice exposed for 28 days and allowed a 34 or 35 day treatment-free expression time. Based on these considerations, furfural did not induce an *in vivo* mutagenic response in this transgenic mouse test system.

Overall Conclusions for Furfural

Furfural has been studied in a comprehensive battery of acceptable genetic toxicology assays, many of which were sponsored by the NTP, provided valuable information, and are acceptable for regulatory purposes. Furfural is uniformly negative in bacterial assays for gene mutations but is mutagenic in cultured mammalian cells (mouse lymphoma). It is also clastogenic and induces SCEs in cultured CHO cells as well human lymphocytes. It is of note that the genotoxic response is more apparent in the absence rather than the presence of exogenous rodent metabolic activation. This observation is consistent with the negative findings from assays in whole animals regardless of the genetic endpoint examined (e.g., chromosome aberrations and SCEs in mouse bone marrow or gene mutations in transgenic mice) as well as the negative SCE results found in worker occupationally exposed to furfural. Overall, the data suggest that while furfural has intrinsic mutagenic potential in cultured mammalian cells, it is not expressed in whole animals since it is rapidly metabolized by the liver and rendered either non-mutagenic or markedly less mutagenic. Additionally, the negative data for the *in vivo* gene mutations assay, which examined the mouse liver as the target for furfural-induced tumorigenic activity, rule out mutagenicity as a possible mode of action for the induction of liver tumors seen in the 2-year mouse bioassay. Based on these considerations, there is no concern for mutagenicity.

B. Furfuryl Alcohol

The hydrogenated product of high pressure reduction of furfural, furfuryl alcohol was also assayed in a series of genetic toxicology studies sponsored by the NTP; summaries are presented below:

In Vitro Studies

Gene Mutation

The negative results of the NTP-sponsored *S. typhimurium* mammalian microsome mutagenicity assay conducted by Mortelmans *et al*. (1986) are discussed above with furfural.

Monien et al., (2011) found furfuryl alcohol to be mutagenic in *Salmonella typhimurium* TA100 strains engineered to express human sulfotransferase (TA100 SULT1A1*1 or TA100 SULT1A1*1Y), which is similar to the cytosolic protein level found in human liver (i.e., 0.3% or 2.6%, respectively). Additionally, the investigators found evidence of two DNA adducts [N^2-((furan-2-yl) methyl)-2' –deoxyguanosine (N^2-MFdG) and N^6-((furan-2-yl) methyl)-2' – deoxyadenosine (N^6-MFdA) in the bacterial DNA. Increases in revertant colonies were 2- and 4-fold higher than background for the strain showing the lower expression (TA100 SULT1A1*1) and 4- and 7-fold for the strain with the higher expression (TA100 SULT1A1*1Y) at 25 or 100 nmol/plate furfuryl alcohol, respectively. The investigators further reported that these findings correlated with the occurances of these adducts in the liver, lungs, and kidney of FVB/N mice receiving ≈390 mg/kg/day of furfuryl alcohol in drinking water for 28 days.

The findings of these investigation should be viewed with caution for several reasons: 1) there is little or no information on the bacterial strain characteristics, (*e.g.*, no historical control data); 2) the data should be confirmed in an independent study; 3) no primary data were presented; therefore, the presentation of means and standard errors instead of standard deviations for the bacterial mean values does not allow an independent assessment of variation around the means, 4) no primary data were presented for DNA adducts in the liver , kidney or lung of male mice, 5) findings of N^2-MFdG or N^6-MFdA adducts did not correlate with the induction of tumors at specific sites in the NTP mouse bioassay (e.g., similar levels of adducts were reported in the mouse liver, lung and kidney; however, the only site of tumor formation in the lifetime inhalation bioassay mouse bioassay was the renal tubules. Furthermore, the dose at which adducts were detected was higher than the established maximum tolerated dose (60 mg/kg/day) and the tumorigenic dose for the NTP study. Based on these considerations, CARC concluded that these data do not provide reliable evidence of an *in vivo* genotoxic response.

Chromosome Aberrations

NTP also sponsored an *in vitro* mammalian cell chromosome aberration assay with furfuryl alcohol. In this study, Stich *et al.* (1981), exposed Chinese hamster ovary (CHO) cells to 0, 160, 300 or 500 µg/mL -S9 for 10 hours or 0, 300, 500 or 1000 µg/mL +S9 for 2 hours. Cultures were harvested at 12- 13 hours after treatment. Metaphase analysis revealed that in the absence of S9 activation, furfuryl alcohol was not clastogenic. With S9, the percentage of cells with chromosome aberrations was significantly ($p<0.05$) increased at 500 and 1000 µg/mL but the increase was not dose related or reproduced in a repeat trial.

Other Mutagenic Mechanisms

An *in vitro* investigation of sister chromatid exchanges (SCEs) in CHO cells was sponsored NTP and was conducted by Stich *et al*. (1981). In this study, CHO cells were exposed to 0, 16, 50, 160 or 500 µg/mL -S9 for 26 hours (Trial 1) or comparable non-activated concentrations for 26 hours (Trial 2) or 0, 16, 50, 160 or 500 µg/mL +S9 for 2 hours. Cells were processed and second division metaphases were analyzed. The non-activated test material at 500 µg/mL induced a positive increase in the percentage SCEs/chromosomes in Trial 1. This result was confirmed in Trial 2 with positive effects at 160 and 500 µg/mL -S9. By contrast, the S9-activated test material was not genotoxic.

The negative findings of the SCE study of Gomez-Arroyo and Souza (1985), with cultured human lymphocytes or lymphocytes collected from workers occupationally exposed to furfural or furfuryl alcohol, were previously discussed with furfural.

In Vivo Studies

In the NTP *in vivo* mouse bone marrow cytogenetic assays, furfuryl alcohol was not clastogenic in the bone marrow cells of male B6C3F1 mice harvested 17 hours after administration of 0, 75, 150 or 300 mg/kg by intrapertoneal injection. In bone marrow cells harvested at 36 hours after exposure to 0, 50, 100 or 200 mg/kg, no significant pairwise increases were observed but a significant ($p<0.05$) trend in cells with chromosome aberrations was seen. However, these results were not reproduced in two subsequent independent trials using comparable test material doses and a comparable harvest time. It was, therefore, concluded that furfuryl alcohol was not clastogenic in whole animals. A similar conclusion was drawn for the mouse bone marrow micronucleus assay performed with 6 groups of 5 male B6C3F1 mice dosed intraperitoneally with 0, 15.625, 31.25, 62.5, 125, or 250 mg/kg furfuryl alcohol/day for 3 consecutive days. Finally, furfuryl alcohol did not induce an increase in SCE in mouse bone marrow cells of male B6C3F1 mice receiving 0, 75, 150 or 300 mg/kg and sacrificed at 23 hours post-treatment or at 0, 37.5, 75 or 150 mg/kg and harvested at 23 hours post-treatment.

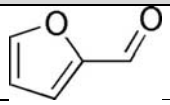
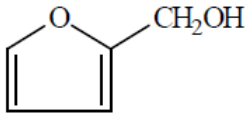
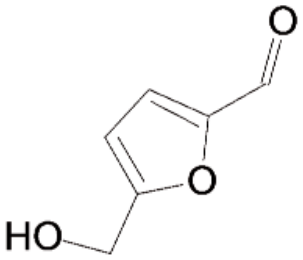
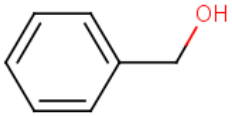
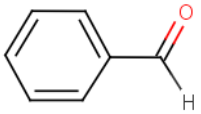
Overall Conclusions for Furfuryl Alcohol

Furfuryl alcohol has been studied in a battery of acceptable genetic toxicology assays, many of which were sponsored by the NTP, provided valuable information, and are acceptable for regulatory purposes. The data indicate that furfuryl alcohol is not mutagenic in bacteria and does not cause chromosome aberrations or SCE induction in mammalian cells. These *in vitro* data are supported by the results of whole animals studies showing that furfuryl alcohol was not clastogenic, aneugenic or genotoxic in mouse bone marrow cytogenetic, micronucleus or SCE assays. It is, therefore, concluded that furfuryl alcohol does not present a mutagenic concern.

3. Structure-Activity Relationship

The available literature was screened for relevant information on structural analogs of furfural and furfuryl alcohol. The chemical name, CAS registry number and structure of the analogs identified are listed in Table 25. A brief discussion of the mutagenicity and carcinogenicity study results from NTP are provided.

Table 25. Furfural, furfuryl alcohol and their structural analogs.

Chemical Name	CASRN	Structure
Furfural	98-01-1	
Furfuryl alcohol	98-00-0	
Furan	110-00-9	
5-(Hydroxymethyl)-2-furfural	67-47-0	
Benzyl Alcohol	100-51-6	
Benzaldehyde	100-52-7	

Furan was negative in the Ames assay and did not induce unscheduled DNA synthesis in rat or mouse hepatocytes following treatment *in vitro* or *in vivo*. Furan did induce gene mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells in the absence of metabolic activation and was observed to induce chromosomal aberrations at 250 mg/kg bw in the bone-marrow cells of B6C3F1 male mice injected intraperitoneally, when an extended protocol involving a late harvest time was employed. **Furan is classified as a Group 2B carcinogen (possibly carcinogenic to humans) by IARC (IARC, vol 63). There is sufficient evidence of carcinogenicity in animals with cholangiocarcinomas observed in the liver and mononuclear cell leukemia (NTP, 1993).**

5-(Hydroxymethyl)-2-furfural (5-HMF) was weakly mutagenic in *S. typhimurium* strain TA 100 in the absence of exogenous metabolic activation in one study. In the second study by the NTP, no mutagenicity was detected with or without activation in any strain. In an oral gavage chronic/carcinogenicity study of 5-(hydroxymethyl)-2-furfural by NTP, the NTP concluded that there was no evidence of carcinogenic activity in male or female rats or in male $B6C3F_1$ mice. **There was some evidence of carcinogenic activity for 5-HMFin $B6C3F_1$ female mice based on increased incidences of hepatocellular adenomas (EFSA, 2011).**

Benzyl Alcohol was not mutagenic in *S. typhimurium* in the presence or absence of exogenous metabolic activation. In the mouse L5178Y/TK+/- lymphoma assay, benzyl alcohol induced an increase in triflurothymidine (Tft)-resistant cells in the absence, but not in the presence, of S9; the effect was associated with toxicity. With Chinese hamster ovary (CHO) cells, an equivocal increase in sister chromatid exchanges both with and without S9 and a significant increase in chromosomal aberrations in the presence of S9 (but not the absence) was observed. In an oral gavage chronic/carcinogenicity study of benzyl alcohol conducted by NTP there was no evidence of carcinogenic activity in male or female F344/N rats or in male or female $B6C3F_1$ mice.

Benzaldehyde was not mutagenic in *S. typhimurium* and did not induce chromosomal aberrations in CHO cells, with or without exogenous metabolic activation. Benzaldehyde induced increases in triflurothymidine-resistant mouse lymphoma cells in the absence of exogenous metabolic activation and increased sister chromatid exchanges in CHO cells in both the presence and absence of metabolic activation. In an oral gavage chronic/carcinogenicity study of benzaldehyde conducted by NTP, there was no evidence of carcinogenic activity in male or female F344/N rats at doses up to 400 mg/kg per day and **there was some evidence of carcinogenic activity in male or female $B6C3F_1$ mice, as indicated by increased incidences of squamous cell papillomas and hyperplasia of the forestomach.**

Being a direct-acting reactive electrophilic chemical, furfural is expected to have a greater potential for inducing cancer by the inhalation route than the oral route because of the portal-of-entry effect. A structurally related chemical, formaldehyde, is a well known carcinogen via the inhalation route but does not seem to be a serious cancer concern by the oral route unless there is a massive exposure to overwhelm the detoxification capacity. There is some suggestive evidence that the nasal carcinogenic effect of furfuryl alcohol via the inhalation route may be related to its oxidation to furfural as the proximate or ultimate carcinogen.

4. Subchronic and Chronic Toxicity

The primary target organ for furfural exposure via the oral route is the liver while the primary target organ for exposure via the inhalation route is the nasal cavity. In two independent studies conducted under the U.S. National Toxicology Program (NTP), Furfural (99% a.i.; Lot # Q112979) was administered for up to 13 weeks in corn oil via gavage to 10 F344/N rats/sex/group at nominal dose levels of 0, 11, 22, 45, 90 or 180 mg/kg/day or 10 $B6C3F_1$ mice/sex/group at 0, 75, 150, 300, 600, or 1200 mg/kg/day. The dosages were administered daily 5 days/week at dose volumes of 5 mL/kg in the rats and 10 mL/kg in the mice. Survival, body weight, body weight gain, and organ weight data were provided. Histopathology liver findings were summarized in the text (MRID 46011015).

In the rat study, 9/10 males and 10/10 females in the 180 mg/kg group, and 1/10 males and 4/10 females in the 90 mg/kg group died before the end of the study. The majority of the 90 mg/kg deaths were due to gavage injury. Mean body weights and body weight gains were similar to controls; terminal body weights were only slightly increased ($p \leq 0.05$) in the 45 and 90 mg/kg males compared to controls. In the 90 mg/kg male rats, increases (p less than or equal to 0.05) in absolute and relative (to body) liver weights were observed. A non-dose dependent increase in the incidence of minimal to mild hepatocyte cytoplasmic vacuolization was observed in controls and all treated males (4/10, 10/10, 10/10, 10/10 and 9/10 at 0, 11, 22, 45, and 90 mg/kg, respectively). Based on this study, the NTP selected 60 mg/kg/day as the high dose and 30 mg/kg/day as the low dose for the subsequent two year rat study. The Systemic Toxicity NOAEL is 45 mg/kg/day and the Systemic Toxicity LOAEL is 90 mg/kg/day based on liver weight changes and liver pathological observations. The observation data available in this study for endpoint determination was minimal; this study was used as a range-finding study for the NTP carcinogenesis study.

In the mouse study, all animals that received 1200 mg/kg and the majority of the 600 mg/kg group died within the first few weeks of the study. These deaths were considered treatment-related. At 150 and 300 mg/kg, mean body weights, body weight gains, and terminal body weights were slightly decreased in the males and were similar to controls in the females. Increased (p less than or equal to 0.05) relative (to body) liver weights were observed in the 300 mg/kg males and the 75, 150, and 300 mg/kg females. It was stated that centrilobular hepatocyte coagulative necrosis was observed in the 1200 mg/kg group (8/10 males and 2/10 females) and in males at 600 mg/kg (9/10), 300 mg/kg (1/10), and 150 mg/kg (1/10). Inflammation, characterized by a minimal to mild mononuclear inflammatory cell infiltrate, was also observed in the presence of liver necrosis. Based on this study, the NTP selected 175 mg/kg/day as the high dose and 50 mg/kg/day as the low dose for the subsequent mouse carcinogenicity study. The Systemic Toxicity NOAEL is less than 75 mg/kg/day and the Systemic Toxicity LOAEL is equal to or greater than 75 mg/kg/day based on liver weight changes and liver pathological observations. The observation data available in this study for endpoint determination were minimal. This study was used as a range-finding study for the NTP carcinogenesis study.

In a subchronic inhalation toxicity study (MRID#'s 46426504 and 46426505), furfural (99% a.i.) commercially obtained from Sigma/Aldrich, Brussels, was administered as a vapor by the nose-only inhalation route to 5 rats/sex/group (Fischer 344 strain) initially to concentrations 0, 40, 80, 160, 320, 640, and 1280 for 6 hours per day, 5 days per week for 28 weeks. These groups were designated as Groups A to G, respectively. Additional treatment groups exposed to periods of 3 hours/day (5/sex/group) were exposed to furfural vapors at 320, 640 and 1280 mg/m^3, 5 days per week for 28 days. These groups were designated as H, I and J, respectively. Because of excessive mortalities in groups F, G and J, this design was changed. Group F (640 mg/m^3) was discontinued and two new groups with fresh animals were set up: 20 mg/m^3.for 6 hour exposures, designated herein as G2; 160 mg/m^3 for 3 hour exposure periods, designated as J2.

The inhalation treatment groups were evaluated daily for toxicity, weekly for body weight and food consumption, terminally for hematology changes, clinical chemistry and gross and histopathological effects. Group F (640 mg/m^3) was dropped after deaths occurred during day 1 and day 8. All animals exposed to concentrations of 1280 mg/m^3 whether for 6 hours (Group G) or for 3 hours, Group J, died in the first day of exposure. These groups were reconstituted at lower concentrations and designated G2 and J2 as noted above. There was no additional mortality in the revised dosing treatments for the rest of the study.

Body weight, food consumption, and clinical pathology were not adversely affected by the inhalation treatments. Pathological changes were seen in the nasal epithelium, some seen effecting all animals at all treatment levels. Other effects were generally dose related. Treatment related pathological effects were limited to olfactory and respiratory epithelium of the nasal cavity. There were no treatment related effects on the kidney, liver, spleen and thymus pathology. Respiratory epithelial atypical hyperplasia was seen in all treated males and females (5/5) for 6 hour exposure groups 20 mg/m^3 to 320 mg/m^3 (Groups G2, B, C, D, and E) and 3 hour exposure groups of 160 mg/m^3 to 640 mg/m^3 (Groups J, H and I). Respiratory epithelial squamous metaplasia was also found in all males and female (5/5) for the same 6 hour exposure groups (G2, B, C, D and E) and all of the females (5/5) for the 3 hour exposure groups (J2, H and I) and 3-4/5 males in the same 3 hour exposure groups. Respiratory epithelial squamous metaplasia and atypical hyperplasia were seen in males and females in a suggestive dose-response from the lowest concentration to the higher ones. Thus there were no inhalation treatments that did not result in nasal epithelium damage; however, the severity of the damage was noted to be less intense in the 3 hour exposure groups compared to the 6 hour exposure groups of animals. The Systemic Toxicity LOAEL is 20 mg/m^3 (the lowest dose tested) based on nasal epithelial pathology seen throughout all of the treated animal groups. There was no Systemic Toxicity NOAEL established.

5. Mode of Action Studies

Data to support a mode of action (MOA) analysis for the liver tumors in furfural treated animals and the liver and nasal tumors in furfuryl alcohol treated animals were not provided.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE EVIDENCE

Furfural

Furfural in corn oil was administered by gavage to F344/N rats (50/sex/dose) at dose levels of 0, 30, or 60 mg/kg bw/day, 5 days/week for 2 years (104 weeks). Furfural in corn oil was administered by gavage to B6C3F1 mice (50/sex/dose) at dose levels of 0, 50, 100, or 175 mg/kg bw/day, 5 days/week for 2 years (104 weeks).

The CARC considered the following for a weight-of-evidence determination of the carcinogenic potential of furfural.

Evidence for Carcinogenicity

Rat

Cholangiocarcinomas of the liver: Two male rats at the high dose group (60 mg/kg/day) had this rare tumor. The incidence (4%), although not statistically significant, was outside of the historical control level (0.4% ± 0.88%) for this tumor type. In addition, these lesions were corroborated by the presence of non-neoplastic lesions (bile duct dysplasia with fibrosis and centrilobular necrosis) in male rats at the high dose. Bile duct dysplasia with fibrosis is considered to be an early stage in the development of cholangio-carcinomas. The concern for the presence of this tumor was enhanced by the occurrence of this tumor (cholangiocarcinoma) in male rats exposed to Furan, a structurally related compound. **The CARC considered the cholangiocarcinomas of the liver in male rats to be treatment-related.**

Adequacy of Dosing: The doses tested were considered to be adequate, but not excessive, in both sexes to assess the carcinogenic potential of furfural. This was based on the results of the 16-day and 13-wee studies, and the presence of non-neoplastic histopathological liver lesions in males in this study.

Mouse

Liver Tumors: Male mice had statistically significant trends, and statistically significant pair-wise comparisons of the high dose (175 mg/kg/day) with the controls, for liver adenomas, carcinomas, and adenomas and carcinomas combined (all at $p < 0.01$). Female mice had significant trends for liver adenomas, and liver adenomas and/or carcinomas combined (at $p < 0.01$). There was a significant pair-wise comparison of the 175 mg/kg/day dose group with the controls for liver adenomas (at $p < 0.05$). All of these tumors are outside of the reported historical control incidence. These tumors were corroborated by the presence of non-neoplastic lesions (chronic inflammation and increased pigmentation of the liver) in both male and female mice. The concern for the presence of these tumors was enhanced by the occurrence of this liver tumor in $B6C3F_1$ female mice exposed to 5-methylflurfural and marked increases of hepatocellular

neoplasms in each sex of mice exposed to benzofuran, structurally related compounds. **The CARC considered the liver tumors in male and female mice to be treatment-related.** In males, there was also an increased incidence of kidney tumors in treated groups compared to controls; however, there were no statistically significant trends or pairwise comparisons for the tumor findings. **The CARC did not consider the kidney tumors in males to be treatment-related.** In females, there was a statistically significant trend for forestomach squamous cell papillomas, no statistically significant pairwise comparisons were observed. Although the incidence was outside of the historical control range, **the CARC did not consider the forestomach tumors to be of toxicological significance since the tumorscould be due to the irritating effect of gavage administration, none of the animals had malignant lesions of the forestomach and these tumors are not relevant for humans.**

Adequacy of Dosing: The highest dose tested was considered to be adequate, but not excessive, in both sexes, to assess the carcinogenic potential of furfural. This was based on the presence of non-neoplastic histopathological liver lesions (chronic inflammation and liver pigmentation) in males and females, and the results of the subchronic study.

Furfuryl Alcohol

Furfuryl alcohol was administered via inhalation to F344/N rats (50/sex/dose) at concentrations of 0, 2, 8, or 32 ppm for 6 hours/day, 5 days/week for 2 years (105 weeks). Furfuryl alcohol was administered via inhalation to B6C3F1 mice (50/sex/dose) at concentrations of 0, 2, 8, or 32 ppm, 5 days/week for 2 years (104 weeks)

The CARC considered the following for a weight-of-evidence determination of the carcinogenic potential of furfuryl alcohol.

Evidence for Carcinogenicity

Rat

Nasal tumors: Male rats had a statistically significant trend for nasal epithelial squamous cell carcinomas at $p<0.05$. There was also a significant trend at $p<0.01$, and a significant pair-wise comparison of the high dose group with the controls at $p<0.05$, for combined nasal lateral wall adenomas, epithelial adenomas, epithelial carcinomas and epithelial squamous cell carcinomas in male rats at the highest concentration (32 ppm). These tumors were corroborated by the presence of non-neoplastic lesions (hyperplasia of the nasal lateral wall, atrophy and metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium) which were observed in male and female rats in the 2-year study. These lesions increased in incidence and severity with increasing concentration. **The CARC considered the nasal tumors in male rats to be treatment-related. The CARC did not consider the nasal tumors and renal tumors observed in females to be treatment related due to the low incidences, lack of dose response relationship, absence of corroborative non-neoplastic lesions and lack of statistical significance.**

Adequacy of Dosing: The highest dose tested was considered to be adequate, but not excessive, in both sexes to assess the carcinogenic potential of furfuryl alcohol. This was based on the presence of non-neoplastic histopathological lesions in the nose (hyperplasia of the lateral wall, atrophy and metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium) in males and females, and the results of the subchronic study. Although there was 100% mortality at the high dose in the main study, the CARC did not consider this concentration to be excessive, since an adequate number of rats were available for data evaluation.

Mouse

Kidney Tumors: Male mice had statistically significant trends for renal tubule adenomas at $p < 0.05$. There was also a significant trend at $p < 0.01$, and a significant pair-wise comparison of the 32 ppm dose group with the controls at $p < 0.05$, for renal tubule adenomas and carcinomas combined. Ad hoc analyses were run on the male mouse kidney tumors because there were no individual animal data provided for the step sectioning performed on this tissue. These tumors were corroborated by the presence of non-neoplastic lesions (nephropathy and renal tubule degeneration) in both male and female mice in the cancer study. The severity of the lesions increased with increasing dose in male mice only. **The CARC considered the kidney tumors in male mice to be treatment-related.**

Adequacy of Dosing: The highest dose tested was considered to be adequate, but not excessive, in both sexes to assess the carcinogenic potential of furfuryl alcohol. This was based on the results of the 16-day and 13-week studies and the presence of non-neoplastic histopathological lesions in the kidney (nephropathy and renal tubule degeneration) in males in the cancer study.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC classified **furfural as "Likely to Be Carcinogenic to Humans."** This determination was based on the following:

(i) Treatment-related cholangiocarcinoma of the liver, a rare tumor type, observed in male rats;
(ii) Treatment-related liver tumors (adenomas, carcinomas and/or combined adenomas/carcinomas) observed in male and female mice;
(iii) Occurrence of hepatocellular neoplasms in each sex of mice with compounds structurally very similar to furfural; and

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC classified **furfuryl alcohol as "Likely to Be Carcinogenic to Humans."** This determination was based on the following:

(i) Treatment-related nasal tumors (adenomas, carcinomas and/or squamous cell carcinomas observed in male rats. ;
(ii) Treatment-related kidney tumors (adenomas, carcinomas and/or combined adenomas/carcinomas observed in male mice;

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC recommended quantification of human cancer risk using a linear approach since no mode of action data are available to support a non-linear mode of action for the tumor types seen with furfural and furfuryl alcohol.

VIII. CARC RECOMMENDATIONS

Based on the toxicological database, it appears that furfural may be more toxic via the inhalation route of exposure. Therefore, the CARC recommends that an inhalation carcinogenicity study be conducted for furfural.

IX. REFERENCES

European Food Safety Authority (2011). Scientific Opinion on Flavouring Group Evaluation 218, Revision 1 (FGE.218Rev1) : alpha,beta-Unsaturated aldehydes and precursors from subgroup 4.2 of FGE.19 : Furfural derivatives. EFSA Journal 9(3) :1840.

Gomez-Arroyo, S and Souza, VS (1985). *In vitro* and occupational induction of sister-chromatid exchanges in human lymphocytes with furfuryl alcohol and furfural. Mutat Res 156: 233-238.

Irwin, R. (1990). NTP Technical report on the toxicology and carcinogenesis studies of furfural in F344/N rats and B6CF1mice (gavage studies). NIH publication number 90-2837, National Toxicology Program.

Marnett, LJ, Hurd, HK, Hollstein, MC, Levin, DE, Esterbauer, H, Ames, BN. (1985). Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. Mutat Res 148:25-34.

MCGregor, DB, Brown, A, Cattanach, P, Edwards, I, McBride, D, Caspary, WJ. (1988). L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay II: 18 coded compounds. Environ Mol Mutagen 11: 91-118.

Monien, BH, Herrmann, K, Florian, S, Glatt, H. (2011). Metabolic activation of furfuryl alcohol: formation of 2-methylfuranyl DNA adducts in Salmonella typhimurium strains expressing human sulfotransferase 1A1 and in FVB/N mice. Carcinogenesis. 32:1533-1539.

Moore, M, Honma, M, Clements, J, Bolcsfoldi, G, Cifone, M, Delongchamp, R *et al*. (2002). Mouse lymphoma thymidine kinase mutation assay: International workshop on genotoxicity tests workgroup report—Plymouth, UK 2002. Mutat Res 540:127-140.

Mortelmans, K, Haworth, S. Lawlor, T, Speck, W. Tainer, B. Zeiger, E. (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ Mutagen 8 (Suppl 7), 1-119.

NTP (National Toxicology Program). 1990. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Furfural (CAS No. 98-01-1) in F344/N rats and B6C3F1 mice (Gavage Studies). NTP-TR-382, MRID 46011016.

NTP (National Toxicology Program). 1999. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Furfuryl Alcohol (CAS No. 98-00-0) in F344/N rats and B6C3F1 mice (Inhalation Studies). NTP-TR-482, MRID 49161601.

NTP (National Toxicology Program). 1993. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Furan (CAS No. 110-00-9) in F344/N rats and B6C3F1 mice (Gavage Studies). NTP-TR-402.

Stich, HF, Rosin, MP, Wu, CH, Powrie, WD. (1981). Clastogenicity of furans found in food. Cancer Lett. 13:89-95.

Sarfaraz, S and Katz AC. (2013). Volume 1-Furfural and Furfuryl Alcohol: Summary of Weight of Evidence from Mutagenicity Studies. toXcel, LLC. Unpublished. MRID 49262201.

Woodruff, RC, Mason, JM, Valencia, R, Zimmering, S. (1985). Chemical mutagenesis testing in Drosophila. V. Results of 53 coded compounds tested for the National Toxicology Program. Environ Mol Mutagen 7:677-702.